

**CLAIMS**

What is claimed is:

1     1.     An automated method for the measurement of residual protein  
2     in a cellular specimen, comprising:

3             (a)    providing a plurality of stained subsamples from a  
4     cellular specimen;

5             (b)    automatically selecting a Z position in each subsample  
6     for imaging a candidate object of interest;

7             (c)    automatically obtaining a low magnification image of  
8     the candidate objects of interest comprising obtaining a  
9     plurality of pixels in each subsample;

10            (d)    automatically filtering the candidate object of  
11     interest pixels in each subsample with a low pass filter;

12            (e)    automatically morphologically processing the candidate  
13     object of interest pixels in each subsample to identify artifact  
14     pixels;

15            (f)    automatically identifying the candidate object of  
16     interest in each subsample by eliminating pixels identified as  
17     artifact pixels;

18            (g)    adjusting the apparatus to a higher magnification;

19            (h)    automatically acquiring a higher magnification image of  
20     the subsample, at the location coordinates corresponding to the

low magnification image, for each candidate object of interest identified in (f);

(i) automatically transforming pixels of the higher magnification image in a first color space to a second color space to differentiate higher magnification candidate object of interest pixels from background pixels;

(j) automatically identifying, at high magnification, an object of interest from the candidate object of interest pixels in the second color space; and

(k) automatically determining the optical density of the protein in a cell contained in a subsample, wherein the optical density is indicative of the residual component of a cellular protein.

2. The method of claim 1, wherein the first color space comprises red, green, and blue components for each pixel and the transforming step includes converting the red, blue and green components for each pixel in the first color space to pixel values in a hue, saturation, and intensity space.

3. The method of claim 2, wherein the hue, saturation, and intensity pixel values are compared to a threshold to

42        identify pixels having a component value equal to or greater  
43        than said threshold as candidate object of interests pixels.

44        4.     The method of claim 1, wherein the cellular protein is an  
45        enzyme.

46        5.     The method of claim 4, wherein the enzyme is alkaline  
47        phosphatase (AP).

48        6.     The method of claim 4, wherein the enzyme is acid  
49        phosphatase (AcP).

50        7.     The method of claim 4, wherein the enzyme is "-naphthyl  
51        butyrate esterase.

52        8.     The method of claim 1, wherein the cellular protein is  
53        assayed immunologically.

54        9.     The method of claim 1, wherein the image is a color image.

55        10.    The method of claim 1, wherein the image is a digital image.

56

57 11. A computer program, residing on a computer-readable medium,  
58 for obtaining images of subsamples of a cellular specimen,  
59 the computer program comprising instructions for causing a  
60 computer to:

61 (a) select a Z position for imaging a candidate object of  
62 interest in a subsample;

63 (b) obtain a low magnification image of the candidate  
64 object of interest comprising obtaining a plurality of pixels;

65 (c) filter the candidate object of interest pixels in each  
66 subsample with a low pass filter;

67 (d) morphologically process the candidate object of  
68 interest pixels in each subsample to identify artifact pixels;

69 (e) identify the candidate object of interest by  
70 eliminating pixels identified as artifact pixels;

71 (f) adjust the apparatus to a higher magnification;

72 (g) acquire a higher magnification image of the subsample,  
73 at the location coordinates corresponding to the low  
74 magnification image, for each candidate object of interest  
75 identified in (e);

76           (h) transform pixels of the higher magnification image in a  
77 first color space to a second color space to differentiate higher  
78 magnification candidate object of interest pixels from background  
79 pixels;

80           (i) identify, at higher magnification, an object of  
81 interest from the candidate object of interest pixels in the  
82 second color space; and

83           (j) score a protein level in the subsample by determining  
84 the optical density of the protein in a cell.

1       12. An automated method for the measurement of residual protein  
2 in a cellular specimen, comprising:

3           (a) providing a plurality of stained subsamples from a  
4 cellular specimen;

5           (b) automatically selecting a Z position in each subsample  
6 for imaging a candidate object of interest;

7           (c) automatically obtaining a low magnification image of  
8 the candidate objects of interest comprising obtaining a  
9 plurality of pixels in each subsample;

10          (d) automatically filtering the candidate object of  
11 interest pixels in each subsample with a low pass filter;

12           (e) automatically morphologically processing the candidate  
13 object of interest pixels in each subsample to identify artifact  
14 pixels;

15           (f) automatically identifying the candidate object of  
16 interest in each subsample by eliminating pixels identified as  
17 artifact pixels;

18           (g) adjusting the apparatus to a higher magnification;

19           (h) automatically acquiring a higher magnification image of  
20 the subsample, at the location coordinates corresponding to the  
21 low magnification image, for each candidate object of interest  
22 identified in (f);

23           (i) automatically transforming pixels of the higher  
24 magnification image in a first color space to a second color  
25 space to differentiate higher magnification candidate object of  
26 interest pixels from background pixels;

27           (j) automatically identifying, at high magnification, an  
28 object of interest from the candidate object of interest pixels  
29 in the second color space; and

30           (k) automatically identifying cells contained in a  
31 subsample and automatically determining the optical density of  
32 the protein in a cell contained in an identified cell, wherein

33 the optical density is indicative of the residual component of a  
cellular protein.